MATING-TYPE EFFECT ON *CIS* MUTATIONS LEADING TO CONSTITUTIVITY OF ORNITHINE TRANSAMINASE IN DIPLOID CELLS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Cis-acting regulatory mutations have been isolated that affect L-ornithine transaminase (OTAse), an enzyme catalyzing the second step of arginine breakdown in yeast. These mutations lead to constitutive synthesis of OTAse at various levels. Two different types of mutations have been recovered, both of which are tightly linked to the structural gene (*cargB*) for this enzyme. One type behaves as a classical operator-constitutive mutation similar to the *cargB*+*O*⁻—*1* mutation previously described (DUBOIS *et al.* 1978).——The second type is peculiar in two respects: the higher level of constitutive OTAse synthesis and the expression of constitutivity in diploid cells. These mutations are designated *cargB*+O^h. They behave as usual operator-constitutive mutations in diploid strains homozygous for mating type (**a**/**a** or α/α), but the constitutivity is strongly reduced in **a**/α diploid cells.

ORNITHINE transaminase (OTAse, L-ornithine-2-oxoacid aminotransferase EC.2.6.1.13), which catalyzes the second step of arginine breakdown to glutamate in yeast (MIDDELHOVEN 1964), has been shown to be induced by L-arginine, as well as by its substrate L-ornithine and their analogs (MIDDELHOVEN 1967; WIAME 1971). Mutations that make OTAse synthesis constitutive have been isolated due to the selective pressure provided by the inability of the biosynthetic *argR*⁻ regulatory mutant to use ornithine as its sole nitrogen source (THURIAUX *et al.* 1968; WIAME 1971). The requirement for an intact *argR* gene *product*, ARGR, for catabolic induction to occur is presumed to result from a necessary interaction between ARGR and the specific catabolic repressor CARGR that has to be removed from the operator loci adjacent to the structural genes coding for arginase and OTAse (WIAME 1971; DUBOIS *et al.* 1978).

Recessive mutations impairing the CARGR catabolic repressor allow both OTAse and arginase to be synthesized constitutively and are unlinked to any structural gene identified so far (WIAME 1971; DUBOIS *et al.* 1978, and unpublished results).

A *cis*-dominant mutation resulting in constitutive OTAse synthesis has been reported to be strongly linked to the *cargB* structural gene and is therefore designated as *cargB*⁺*O*⁻—1 (WIAME 1971; DUBOIS *et al.* 1978).

New *cis*-acting and cargB-linked regulatory mutations responsible for constitutive OTAse synthesis are described in this paper. Some of these define a new genetic class of special interest: their high OTAse level is significantly reduced in diploid cells unless they are homozygous for the mating-type locus.

MATERIALS AND METHODS

A correlation between the notation for genetic markers used in this paper and the standard yeast genetic nomenclature (PLISCHKE *et al.* 1976) is given below.

Notation used in this paper	Notation recommended PLISCHKE *et al.* (1976)	Gene product
cargA	*car1*	arginase (EC.3.5.3.1)
cargB	*car2*	ornithine-oxoacid aminotransferase (EC.2.6.1.13), also called ornithine transaminase (OTAse)
cargA^{+}*O*$^{-}$	*CAR80*	operator
cargB^{+}*O*$^{-}$	*CAR81*	operator
cargR	*car82*	repressor
argRI	*arg80*	repressor
argRII	*arg81*	repressor
argRIII	*arg82*	repressor

Strains: All strains used in this work were derived from the wild-type Σ 1278b (α) and from its mating-type mutant 3962c (**a**). The *argR*$^{-}$ mutations used to provide a genetic background for selection of the strains analyzed in this paper have been described previously (BECHET, WIAME and GRENSON 1965; BECHET, GRENSON and WIAME 1970). They map in three independent loci *argRI*, *argRII* and *argRIII* (BECHET, GRENSON and WIAME 1970). Double mutants *argRI*$^{-}$ *argRII*$^{-}$ were used to avoid selection of revertants that are able to grow on M. ornithine medium. The other *cargB*$^{+}$*O*$^{-}$ and *cargR*$^{-}$ regulatory mutants have been described elsewhere (WIAME 1971; DUBOIS *et al.* 1978).

Growth medium: M medium has been described previously as minimal medium without any nitrogen source (THURIAUX *et al.* 1972). A slight modification has been introduced to avoid lag phases (MESSENGUY 1976) : the trace element solution is sterilized separately and added to medium 165 at the same time as the carbon source and the vitamins. M am medum is M medium supplemented with 0.02 M ammonia as a nitrogen nutrient. Amino acids (0.1%) (w/v) were added when indicated, either to the M medium or to the M am medium. Solid media were prepared by addition of 2% (w/v) agar to the corresponding liquid media.

Mutagenesis: A suspension of exponentially growing cells was spread on solid M ornithine medium at a cell density of about 10^7 cells per plate. Plates were exposed to UV radiation for one min, allowing 50% survival. Fast-growing colonies were selected and tested after purification.

Genetic manipulations: Zygotes resulting from the crosses and spores generated by sporulation of diploid strains were isolated by micromanipulation. Homozygous diploid cells were isolated from the corresponding haploid strains after spontaneous appearance in rich liquid medium. Tetraploid strains were constructed by crossing two diploid strains of opposite mating type (HILGER 1973).

Enzyme assays: Exponentially growing cells were harvested after protein synthesis had been inhibited by addition of cycloheximide (2 μg/ml). Pellets were washed and resuspended in the required buffer. Arginase activity was measured according to MESSENGUY, PENNINCKX and WIAME 1971). Ornithine carbamoyltransferase activity was measured as described by RAMOS *et al.* (1970). Ornithine transaminase activity was measured according to the method of DE HAUWER, LAVALLE and WIAME (1964) as modified by DUBOIS *et al.* (1978).

RESULTS

Selection of strains

Previous results suggest that the amount of OTAse synthesized when L-ornithine is the sole nitrogen source is the rate-limiting step for growth on this medium (unpublished observations). In order to select strains that were as highly constitutive as possible, we decided to retain only fast-growing colonies on M ornithine solid medium after UV mutagenesis (see MATERIALS AND METHODS). Five independent strains appeared to express the expected phenotype. A detailed analysis of each of them supports a distinction between two classes on the basis of how fully constitutive OTAse synthesis is expressed in **a**/α diploid strains.

Characterization of strain JD705

The JD705 mutant appears to belong to the classical operator-type class, previously defined by the *cargB*$^+$*O*$^-$*—1* mutation (DUBOIS *et al.* 1978). Segregant 91E4b bears the isolated *cargB*$^+$*O*$^-$*—2* mutation. The features of this mutation are:

(1) *Constitutive OTAse synthesis.*

(2) *Strong linkage to the structural gene, cargB.* All 27 tetrads analyzed after sporulation of the *cargB*$^+$*O*$^-$*—2/cargB*$^-$ diploid strain were parental ditypes. The *cargB*$^+$ or *cargB*$^-$ genotypes were tested by growth on M ornithine solid medium (print method) and a distinction between *cargB*$^+$*O*$^+$ and *cargB*$^+$*O*$^-$*—2* was possible by measuring OTAse specific activity after growing cells on M am medium (Table 1, Experiment *7 vs.* 1). *cargB*$^+$*O*$^-$*—2* was also crossed with the *cargB*$^+$*O*$^-$*—1* strain, which has been shown to be tightly linked to the structural gene specifying OTAse (DUBOIS *et al.* 1978). Eighteen tetrads were analyzed and no recombinant was found among the segregants, confirming the close linkage between *cargB*$^+$*O*$^-$*—2* and *cargB*. A distinction between *cargB*$^+$*O*$^-$*—1* and *cargB*$^+$*O*$^-$*—2* was possible due to the different level of OTAse constitutive synthesis expressed by these mutants (Table 1, Experiment 7 *vs.* 3).

(3) cis-*dominant expression in diploid cells.* The amount of OTAse synthesized in a **a** *cargB*$^+$*O*$^-$*—2/*α + diploid strain is about half the amount present in the *cargB*$^+$*O*$^-$*—2* haploid strain (Table 1, Experiment 8 *vs.* 7). The same behavior has been reported for the *cargB*$^+$*O*$^-$*—1* mutation (Table 1, Experiment 4 *vs.* 3) and a detailed statistical analysis of similar *cargA*$^+$*O*$^-$ operator mutants has shown that their arginase level is 138 $\pm$15 units in haploid cells as compared to 71 units in diploid cells heterozygous for this allele (DUBOIS *et al.* 1978). In the latter case, a *cargA*$^-$*O*$^-$ rare recombinant is available, which demonstrates the absence of a *trans* effect. These results, together with the linkage test, allow the use of the dilution effect by a factor of two in diploid cells as a rather safe argument in favor of the idea that the *cargB*$^+$*O*$^-$ mutation is *cis* dominant, even though no *cargB*$^-$*O*$^-$ recombinant has yet been obtained.

(4) *Full expression of the* cargB$^+$O$^-$ *mutation in* **a** cargB$^+$O$^-$/α cargB$^+$O$^-$ *diploid cells.* The expected property of **a** *cargB*$^+$*O*$^-$/α *cargB*$^+$*O*$^-$ cells to have

TABLE 1

Generation times on M ornithine medium (30°), OTAse and arginase specific activities in different* cargB+O⁻ *and* cargB+O^h *strains*

Exp. #	Strains	Genotype	Generation time on M orn (in min)	Specific activities on M am OTAse	Arginase
	wild-type strain				
1	Σ 1278b	α +	180	0.019	7.0
	argR⁻ strain				
2	7000a	α *argRI⁻ argRII⁻*	no growth	0.01	3.5
	cargB+O⁻ mutants				
3	7064c	**a** *cargB+O⁻–1*	150	3.8	5.8
4	7064c × Σ 1278b	**a** *cargB+O⁻–1*/α +		1.9	6.5
5	7064c × 7051a	**a** *cargB+O⁻–1*/α *cargB+O⁻–1*		4.1	6.0
6	JD705	α *cargB+O⁻–2 argRI⁻ argRII⁻*	150	1.8	4.0
7	91E4b	α *cargB+O⁻–2*	150	1.8	5.7
8	91E4b × 3962c	α *cargB+O⁻–2*/**a** +		1.0	6.0
9	91E4b × 91E9b	α *cargB+O⁻–2*/**a** *cargB+O⁻–2*		2.0	5.8
	cargB+O^h mutants				
10	JD701	α *cargB+O^h–1 argRI⁻ argRII⁻*	150	10.4	2.9
11	91E3b	α *cargB+O^h–1*	150	10.7	6.1
12	91E3b × 3962c	α *cargB+O^h–1*/**a** +		0.9	6.0
13	91E3b × 91E5a	α *cargB+O^h–1*/**a** *cargB+O^h–1*		1.3	6.4
14	Di 95E6a homozygous diploid	**a** *cargB+O^h–1*/**a** +		4.9	7.0
15	Di 95E6d	α *cargB+O^h–1*/α *cargB+O^h–1*		10.4	6.9
16	JD711	α *cargB+O^h–2 argRI⁻ argRII⁻*	150	10.0	2.0
17	93E7a	α *cargB+O^h–2*	150	10.3	5.2
18	JD734	α *cargB+O^h–3 argRI⁻ argRII⁻*	150	9.8	2.5
19	94E9b	α *cargR+O^h–3*	150	10.6	6.1
20	JD739	α *cargB+O^h–4 argRI⁻ argRII⁻*	150	7.5	2.1
21	95E3d	**a** *cargB+O^h–4*	150	6.8	5.0

* OTAse and arginase specific activities are expressed in micromoles of Δ pyrroline carboxylic acid and urea, respectively, formed per hour per mg of protein.

exactly the same enzyme level as the corresponding haploid cells is nevertheless worth stressing because the major distinction between the *cargB+O⁻* mutants and those belonging to the second type described below is precisely that the latter mutants exhibit significantly reduced expression of constitutive OTAse synthesis in **a**/α diploid cells (Table 1, Experiment 5 *vs.* 3, 9 *vs.* 7, and 13 *vs.* 11).

Characterization of four strains belonging to a new type of cargB-linked constitutive mutation

JD701, JD711, JD734 and JD739 are endowed with constitutive OTAse synthesis at a higher level than the two previously described *cargB+O⁻* mutants (Table 1, Experiments 10, 16, 18 and 20 *vs.* 3 and 7) (see DISCUSSION for a possible implication of this particular feature). This property together with the specificity of these mutations (neither arginase nor ornithine carbamoyltransferase synthesis is affected, as shown in Table 2, Experiment 4 *vs.* 1), and their

TABLE 2

Analysis and specificity of the cargB+O^h *and* cargB+O$^-$ *mutations*

Exp. #	Strain	Genotype	OTAse Specific Activity* M am	OTAse Specific Activity* M am+arg	OTAse Specific Activity* M arg	Arginase Specific activity* M am	Arginase Specific activity* M am+arg	Arginase Specific activity* M arg	OTCase Specific activity* M am	OTCase Specific activity* M am+arg	OTCase Specific activity* M arg
1	Σ 1278b	wild type (+)	0.02	0.4	9.5	7.0	20.3	298	27.1	2.3	1.3
2	7064c	*cargB*$^+$*O*$^-$*-1*	4.0	3.9	8.3	5.2	18.6	291	31.0	2.7	1.5
3	91E4b	*cargB*$^+$*O*$^-$*-2*	1.5	1.5	8.0	5.7	21.5	308	29.0	2.9	1.1
4	91E3b	*cargB*$^+$*O*^{h}*-1*	10.1	10.2	14.0	6.1	21.0	287	35.0	2.5	1.2

* OTAse specific activity is expressed in micromoles of Δ pyrroline carboxylic acid produced per hour per mg of protein. Arginase specific activity is expressed in micromoles of urea produced per hour per mg of protein. OTCase activity is expressed in micromoles of citrulline produced per hour per mg of protein.

tight linkage to the ornithine transaminase structural gene allowed them to be designated as *cargB*$^+$*O*^{h} by comparison with the analogous *cargB*$^+$*O*^{h} mutation (see DISCUSSION) affecting arginase. Each of the four allelic mutants was analyzed in detail, but only one of them is described in this paper because they were all found to behave similarly.

(1) *Linkage between* cargB$^+$O^h—1 *and the* cargB *gene.* Segregant 91E3b harboring the *cargB*$^+$*O*^{h}*—1* mutation isolated from JD701 was crossed with a *cargB*$^-$ strain. Seventeen tetrads were analyzed, among which none was found to be either a tetratype or a nonparental ditype. Here again we could distinguish *cargB*$^+$*O*$^+$ from *cargB*$^+$*O*^{h}*—1* by measuring the OTAse specific activity after growing the cells on M ammonium medium (Table 3, Experiment 3 *vs.* 1). Eight

TABLE 3

Expression of the cargB$^+$O^h *phenotype in diploid and tetraploid cells homozygous and heterozygous for the mating-type locus*

Exp. #	Strain	Genotype	OTAse specific activity on M am medium*	Sporulation ability
1	Σ 1278b	wild type α +	0.019	
2	3962c	wild type **a** +	0.02	
3	91E3b	α *cargB*$^+$*O*^{h}*-1*	10.1	
4	91E5a	**a** *cargB*$^+$*O*^{h}*-1*	10.3	
5	91E3b × 3962c	α *cargB*$^+$*O*^{h}*-1*/**a** +	0.83	+
6	91E3b × 91E5a	α *cargB*$^+$*O*^{h}*-1*/**a** *cargB*$^+$*O-1*	1.3	+
7	Di 91E3b homozygous diploid	α *cargB*$^+$*O*^{h}*-1*/α *cargB*$^+$*O*^{h}*-1*	10.6	—
8	Di Σ 1278b	α +/α +	0.02	—
9	Di 91E5b	**a** *cargB*$^+$*O*^{h}*-1*/**a** *cargB*$^+$*O*^{h}*-1*	10.4	—
10	Di 3962c	**a** +/**a** +	0.02	—
	Segregants from 91E3b × 3962c (one parental ditype among the eight tested)			
11	a	**a** *cargB*$^+$*O*^{h}*-1*	10.2	
12	b	α +	0.1	
13	c	α *cargB*$^+$*O*^{h}*-1*	9.2	
14	d	**a** +	0.09	
	Segregants from 91E3b × 91E5a (all tetrads were parental ditypes)			
15	a	**a** *cargB*$^+$*O*^{h}*-1*	10.2	
16	b	α *cargB*$^+$*O*^{h}*-1*	9.7	
17	c	**a** *cargB*$^+$*O*^{h}*-1*	9.4	
18	d	α *cargB*$^+$*O*^{h}*-1*	10.2	
	Other *cargB*$^+$*O*^{h} strains			
19	93E7a	α *cargB*$^+$*O*^{h}*-2*	10.3	
20	93E7a × 93E7d	α *cargB*$^+$*O*^{h}*-2*/**a** *cargB*$^+$*O*^{h}*-2*	2.8	+
21	94E9b	**a** *cargB*$^+$*O*^{h}*-3*	10.6	
22	94E9b × 94E9c	**a** *cargB*$^+$*O*^{h}*-3*/α *cargB*$^+$*O*^{h}*-3*	1.8	+
23	95E3d	**a** *cargB*$^+$*O*^{h}*-4*	6.8	
24	95E3d × 95E4d	**a** *cargB*$^+$*O*^{h}*-4*/α *cargB*$^+$*O*^{h}*-4*	0.93	+

* OTAse specific activity is expressed in micromoles of Δ pyrroline carboxylic acid produced per hour per mg of protein.

more tetrads resulting from the cross between 91E3b (*cargB*$^+$*O*^{h}—*1*) and 7064c (*cargB*$^+$*O*$^-$—*1*), known to be linked to *cargB*, were examined. No recombinant was found among the segregants, which leads to the conclusion that a strong linkage exists between *cargB*$^+$*O*^{h}—*1* and the structural gene coding for OTAse.

(2) *Expression of the* cargB$^+$O^h—1 *mutation in diploid strains; dependence on the mating genotype of the cells.* The very low OTAse level measured in α *cargB*$^+$*O*^{h}—*1*/**a** + diploid strains obviously means that the constitutive expression of the *cargB*-linked regulatory mutation is greatly reduced in heterozygous diploid cells. Analysis of eight tetrads resulting from the cross between 91E3b (α *cargB*$^+$*O*^{h}—*1*) and the wild-type 3962c is described in Table 3 (Experiment 11, 12, 13, 14 *vs.* 5 and 3). The *cargB*$^+$*O*^{h}—*1* genotype is confirmed for two segregants among the four of each tetrad and the resulting constitutively is high, whether they are of **a** or of α mating type. Still more striking is the very small amount of OTAse synthesized in **a** *cargB*$^+$*O*^{h}—*1*/α *cargB*$^+$*O*^{h}—*1*, as shown in Table 3, Experiment 6. The genotype of this diploid strain is confirmed by tetrad analysis, which reveals that all haploid segregants possess the fully expressed *cargB*$^+$*O*^{h}—*1* phenotype (Table 3, Experiments 15, 16, 17, 18 *vs.* 6). *cargB*$^+$*O*^{h}—*1*/*cargB*$^+$*O*^{h}—*1* diploid cells synthesize amounts of OTAse equal to those obtained in the corresponding *cargB*$^+$*O*^{h}—*1* haploid cells only when they are homozygous for the mating-type locus, whether it is **a** or α (Table 3, Experiments 7 and 9 *vs.* 6).

The assumption that there is a mating-type dependence of the OTAse regulatory mutation is strengthened by the construction of different tetraploid strains followed by the analysis of their segregants (Table 4, Experiments 1 to 34). OTAse synthesis in **a** *cargB*$^+$*O*^{h}—*1*/ **a** + diploid cells is half the level measured in *cargB*$^+$*O*^{h}—*1* haploid cells or in **a** *cargB*$^+$*O*^{h}—*1*/**a** *cargB*$^+$*O*^{h}—*1* diploid cells (Table 4, Experiments 4, 6, 9, 24 *vs.* 1 and 2). This strongly suggests that the *cargB*$^+$*O*^{h} mutation is *cis* dominant (see RESULTS).

DISCUSSION

Two main generalizing aspects arise from the study of the L-ornithine transaminase constitutive mutations reported in this paper:

(1) the *cargB*$^+$*O*^{h} mutations provide another example of a relationship between specific regulatory mutations and the mating genotype. A very similar property has been reported concerning the *cargA*$^+$*O*^{h} mutation affecting arginase synthesis (Wiame and Dubois 1976; Dubois *et al.* 1978).

More recently a highly constitutive strain has been isolated that is affected in the synthesis of both of the enzymes catalyzing urea degradation into NH_3 and CO_2, which are coded for by the closely linked loci *dur1* and *dur2* (Lemoine, Dubois and Wiame, in preparation). Expression of this *durO*^{h} designated mutation in diploid cells is also dependent on their mating type.

These *cis-acting* specific constitutive mutations certainly impair the regulatory loci involved in the classical control mechanism governing the corresponding enzyme synthesis They are tightly linked to the respective structural genes and

TABLE 4

Expression of cargB+O^h *in a genetic background homozygous for the mating-type locus*

Exp. #	Strain	Genotype (abbreviated genetic nomenclature)	OTAse* specific activity	Mating with **a** strains	Mating with α strains	Sporulation ability
	Parental strains					
1	Di 91E3b homozygous diploid	α/α O^h/O^h	10.4	+	—	—
2	Di 3962c × homozygous diploid	**a**/**a** $O+/O+$	0.04	—	+	—
3	Di 93E3b × Di 3962c tetraploid	α α O^h/O^h	1.03	—	—	+
		a/**a** $O+/O+$				
	Segregants from two tetrads (Di 91E3b × Di 3962c)					
4	95E6a	**a**/**a** $O^h/O+$	4.9	—	+	—
5	95E6b	**a**/**a** $O+/O+$	0.05	—	+	—
6	95E6c	α/α $O^h/O+$	4.7	+	—	—
7	95E6d	α/α O^h/O^h	10.4	+	—	—
8	95E7a	**a**/α O^h/O^h	0.98	—	—	+
9	95E7b	α/α $O^h/O+$	4.5	+	—	—
10	95E7c	**a**/**a** $O+/O+$	0.07	—	+	—
11	95E7d	α/**a** $O^h/O+$	0.48	—	—	+
	Segregants from 95E7a (all tetrads were similar to 100E4)					
12	100E4a	α O^h	9.0	+	—	—
13	100E4b	α O^h	10.1	+	—	—
14	100E4c	**a** O^h	9.2	—	+	—
15	100E4d	**a** O^h	10.0	—	+	—
	Segregants from 95E7d (all tetrads were similar to 100E5)					
16	100E5a	**a** O^h	9.9	—	+	—
17	100E5b	**a** $O+$	0.05	—	+	—
18	100E5c	α O^h	10.0	+	—	—
19	100E5d	α $O+$	0.04	+	—	—
	Parental strains					
20	Di 91E5a homozygous diploid	**a**/**a** O^h/O^h	10.8	—	+	—
21	Di Σ 1278b homozygous diploid	α/α $O+/O+$	0.07	+	—	—
	Tetraploid strain					
22	Di 91E5a × Di Σ 1278b	**a**/**a** O^h/O^h	0.93	—	—	+
		α/α $O+/O+$				
	Segregants from a tetrad (Di 91E5a × Di Σ 1278b)					
23	95E8a	**a**/α $O+/O+$	0.06	—	—	+
24	95E8b	α/α $O^h/O+$	5.1	+	—	—
25	95E8c	**a**/**a** O^h/O^h	10.9	—	+	—
26	95E8d	**a**/α $O^h/O+$	0.54	—	—	+
	Segregants from 95E8a (all tetrads were similar to 100E6)					
27	100E6a	**a** $O+$	0.04	—	+	—
28	100E6b	α $O+$	0.03	+	—	—
29	100E6c	**a** $O+$	0.05	—	+	—
30	100E6d	α $O+$	0.04	+	—	—
	Segregants from 95E8d (all tetrads were similar to 100E7)					
31	100E7a	α $O+$	0.04	+	—	—
32	100E7b	α O^h	9.6	+	—	—
33	100E7c	**a** O^h	10.0	—	+	—
34	100E7d	**a** $O+$	0.03	—	+	—

Tetrad analysis after sporulation of the tetraploid cells resulting from the cross between homozygous diploid strains. 95E6a, 95E6c, 95E7b and 95E8b manifest the *cis*-dominant character of the *cargB*+O^h mutation when only one genetic locus specifying the mating type is present in the diploid strain.

* OTAse activity is expressed in micromoles of Δ pyrroline carboxylic acid produced per hour per mg of protein.

addition of inducer does not modify the enzyme level that they confer to the cells. However, the constitutivity is considerably lower in **a**/α O^h/O^h diploid cells than in illegitimate **a**/**a** O^h/O^h or α/α O^h/O^h strains. This particular feature seems to mean that another regulatory phenomenon could be involved as well. Furthermore, the influence of the sexual genotype on specific enzyme regulation does not appear to be restricted to the breakdown of arginine-related compounds. ROTHSTEIN and SHERMAN (1977) recently reported that the derepressed *CYC7–2* mutation (linked to the iso-2-cytochrome *c* structural gene) behaves the same as our O^h strains in legitimate and illegitimate diploid cells.

(2) *cis*-acting constitutive arginase and ornithine transaminase mutations offer the interesting possibility of comparing the levels of enzyme synthesized in both O^- and O^h mutants. All four independent *cargB*$^+O^h$ mutants express a higher constitutivity than the *cargB*$^+O^-$ strains isolated until now. The amount of arginase synthesized in the *cargA*$^+O^h$ strain is also higher than in any of the numerous *cargA*$^+O^-$ mutants ever selected. This common property of O^h mutations could be helpful in attempting to understand the regulatory mechanism involved. Such a comparison is not yet possible for the *dur1-dur2* regulation because no *durO*$^-$ mutation free of **a**/α effect has been isolated so far.

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LITERATURE CITED

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